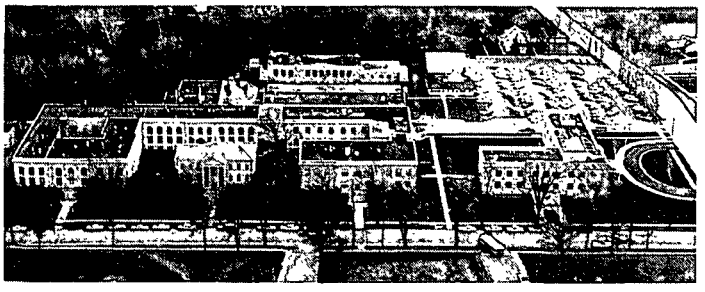


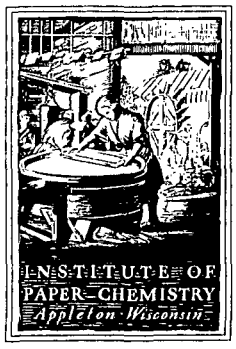
THE INSTITUTE OF PAPER CHEMISTRY, APPLETON, WISCONSIN

Status Report

to the

PROJECT ADVISORY COMMITTEE
ON
FOREST GENETICS

October 28-29, 1986
The Institute of Paper Chemistry
Appleton, Wisconsin 54912



THE INSTITUTE OF PAPER CHEMISTRY
Post Office Box 1039
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October 7, 1986

TO: Members of the Forest Genetics PAC

As indicated in my letter of September 4, I am forwarding a detailed agenda for the fall meeting of our Forest Genetics Project Advisory Committee. Enclosed also is some advance reading material -- a copy of our recent Status Report. Please use these items in preparing for participation.

Preparations here are well underway. New data is being analyzed as collected, talks are being formulated, and slides are being prepared. We have much to present and discuss, including a growing list of publications.

We look forward to your sharing October 28 and 29 with us. Please remember to register, if you have not already done so. Be certain also to circulate the enclosures to other members of your firm who are planning to participate.

Many thanks and best regards.

Sincerely,

Ronald J. Dinus
Director
Forest Biology Division

RJD/ls
Enclosures

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MEETING AGENDA

Forest Genetics

Project Advisory Committee

October 28-29, 1986
The Institute of Paper Chemistry
Continuing Education Center
Appleton, Wisconsin 54912

TUESDAY, OCTOBER 28:

1:00 p.m.	Welcome/Introduction	Dinus
1:15	Somatic Embryogenesis	
	Immature Embryos, Optimum Window	
	Norway and White Spruce (20 min)	Becwar
	Hard Pines, Douglas-fir, and white pine (25)	Wann
	Loblolly Pine Reproductive Development (15)	Becwar
	Mature Seed Embryos (15)	Verhagen
	Plantlet Recovery and Transfer (15)	Becwar
2:45	Coffee Break	
3:00	Biochemistry	
	Characterization of Explants, and Embryogenic and Nonembryogenic Callus (30)	Johnson
	Applications in Conifer Tissue Culture Research (15)	Wann
3:45	Exploratory Research	
	Chloroplast Development (15)	Feirer
	Cellular Origin of Somatic Embryos (15)	Rangaswamy
	Oligosaccharins (15)	Nealey
4:30	Molecular Biology, Recent Developments and Opportunities (30)	Feirer
5:00	Summary, Prognosis and Plans	Dinus
5:30	Cocktails and Dinner	
7:00	Available for Discussion as Desired	

AGENDA (cont'd)

WEDNESDAY, OCTOBER 29:

7:30 a.m.	Breakfast, CEC Dining Room	
8:00	Agenda	Owens/Dinus
8:15	Discussion of Projects	Committee
9:45	Coffee Break	
10:00	Discussion/Deliberations	Committee
11:15	Closing Comments	Owens/Dinus
11:30	Adjournment/Lunch, CEC Dining Room	

Next Meeting: March 30-31, 1987

FOREST GENETICS

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*date of retirement
/bsb
7/15/86

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

Status Report

to the

FOREST GENETICS

PROJECT ADVISORY COMMITTEE

Project 3223

THE MASS PRODUCTION OF CONIFERS

October 28-29, 1986

PROJECT TITLE: The Mass Production of Conifers

PROJECT STAFF: Becwar*, Dinus, Feirer, Johnson, Rangaswamy, Verhagen, Wann*

PRIMARY AREA OF INDUSTRY NEED: Raw Materials

PROGRAM AREA: Increased wood production and quality by somatic embryogenesis and bioengineering

Date: 10/28/86
Budget: $600,000
Period Ends: 6/30/87
Project No.: 3223
Approved by VP-R:

PROGRAM GOAL: Significantly increase forest productivity and quality by mass propagation of superior trees.

PROJECT OBJECTIVE/GOAL:

The overall objective is mass production of genetically improved conifers. The near-term objective is development of procedures for producing plantlets from single cells or small groups of cells.

PROJECT RATIONALE:

Major increases can be obtained in tree growth and quality via clonal propagation of "elite" trees, and creation of new genetic combinations. Planned combinations are difficult to produce with conventional techniques, but are expected to yield individuals having exceptional pest resistance, special qualities, and enhanced site and/or climatic adaptability. Production of plantlets from cell cultures will open the way to securing such gains through genetic engineering. Cell cultures thus form the basis for a second-generation technology, which could greatly accelerate and, possibly, replace existing technology.

RESULTS TO DATE:

Procedures and media have been developed for initiating callus, growing cells in suspension, and maintaining cell lines.

Model systems, including somatic embryogenesis in wild carrot, coffee, and Norway spruce as well as natural embryo development in Douglas-fir, loblolly pine, and aspen, have helped characterize tissue culture requirements, morphology, and biochemical markers. Organogenesis in loblolly pine also was used in evaluating markers. Excised conifer embryos were used to determine nutrient requirements.

Studies with enzyme inhibitors demonstrated involvement of polyamines in embryogenesis, characterized polyamine synthesis pathways in wild carrot and aspen, and indicated that spermidine may be the most important polyamine. Tests with aspen and red pine showed that polyamines play an essential role in natural embryo development.

Numerous cell lines from immature embryos have been and are being monitored to determine if their potential for embryogenesis is greater than those from more mature embryos. Extracts from immature pine seed enhanced wild carrot embryogenesis and increased loblolly pine organogenesis, but had no desirable effects on pine cell suspensions.

*Industrial Research Fellow

Energy charges and ATP levels of both wild carrot and loblolly pine cell suspensions appear adequate to drive biosyntheses. Assays of reducing potential, however, suggest that wild carrot suspensions have better control over their internal workings than do those of pine. Indications are that pine suspensions obtained to date lack sufficient control for organized development.

Somatic embryogenesis in conifers was first observed in Sweden (Norway spruce) and Canada (larch), but has since been achieved in Norway spruce at IPC. Embryogenic larch callus is also being studied. Combining our own approaches with those of others, we now have developed not just a controlled and reproducible procedure, but also one in which embryo numbers can be quantified. A small number of Norway spruce plants have been recovered from embryogenic callus, appear phenotypically normal, and are growing well under greenhouse conditions. The Norway spruce system has been adopted as a model for work on other conifers.

The procedure has since been extended to initiation of embryogenic callus in white spruce as well. Embryogenesis has also been observed in Douglas-fir, albeit at low frequency and not on a reproducible basis. Recently, success was obtained with fair frequency in callus derived from mature Norway spruce seed.

Biochemical and histological work has been facilitated by access to embryogenic and nonembryogenic callus from individual Norway spruce genotypes. Aside from visual and tactile differences, embryogenic callus evolves less ethylene, contains less reductants, and lacks normal chloroplasts as compared to nonembryogenic callus. Such differences have aided development of biochemical markers, are enlarging our understanding of morphogenic processes, and should hasten obtaining embryogenesis in species of more direct interest.

PLANNED ACTIVITY FOR THE PERIOD:

Projected activities for the fiscal year were detailed in a conceptual plan first presented in Progress Report 13, and provided herein as Attachment No. 1. The plan builds upon recent advances in regeneration of conifers from tissue culture, and continues as the guide for course and content of IPC efforts.

The newly developed Norway spruce system remains the model system of choice and is being used in lieu of the former wild carrot system. The "juvenility window" was investigated during the summer months, and work is being continued on modified and new protocols with cones of varying maturity drawn from cold storage. Attempts to obtain the mucilaginous callus that gives rise to somatic embryos in Norway spruce continues in loblolly pine and Douglas-fir. Some have already been produced in the latter species. Efforts with explants from mature Norway spruce seed have been productive, and the effort will be accelerated. Continued success on this front would permit year-round experimentation.

Efforts to develop and apply biochemical markers continue. Ethylene evolution rate is now being used as a primary marker, and others, such as polyamine, reductant, and glutathione status, will be used as secondary markers, if proven useful. Tests of marker utility are done routinely on samples of cultures produced in the course of evaluating explants, media, and growth regulators. Other physical, biochemical, and cytological/histological characteristics remain under investigation for possible use in screening for competence and in enlarging our understanding of developmental processes.

Exploratory work, conducted in support of or ahead of the main effort, is aimed at improving current protocols, incorporating the best of findings from other laboratories, discerning cellular origin of embryogenesis, converting somatic embryos to plants, increasing conversion efficiency, inducing embryogenesis in alternative culture systems, regenerating from single cells and protoplasts, and effecting genetic transformation.

SHORT TERM GOALS:

Goals for the remainder of FY 86-87.

1. Continue efforts to induce embryogenesis in cultures established from stored cones of varying maturities in accordance with findings from summer "juvenility window" work; maximize use of cones in testing and exploiting recent advances by other laboratories; and characterize cultures biochemically to evaluate utility of markers. Emphasize Norway and white spruce, loblolly and other hard pines, Douglas-fir, and white pine.

2. Continue characterizing the Norway spruce model in terms of physical, biochemical, and cytological/histological factors, with the aim of accumulating baseline data and evaluating the utility of primary and other markers. In a similar vein, attempt to characterize the mucilaginous material associated with embryogenic spruce callus, and exudates surrounding nonembryogenic calli. Include other species as appropriate.

3. Optimize procedures for increasing production of somatic embryos per unit of Norway spruce callus, raising efficiency of conversion to intact plants, and recovering plantable material; produce sufficient material for replicated greenhouse trials.

4. Attempt to develop reliable protocols for obtaining embryogenic callus from mature conifer explants; determine characteristics that must be changed to make such explants usable; and evaluate procedures for "rejuvenation."

5. Increase efforts to obtain somatic embryogenesis in Norway spruce using alternative culture systems; emphasize systems more amenable to mass propagation.

6. Execute exploratory research on discerning cellular origin of somatic embryos, regenerating from single cells and protoplasts, effecting genetic transformation, and characterizing embryogenic potential with molecular techniques.

7. Investigate and implement, if possible, procedures for securing immature cones of loblolly pine from South American sources so as to permit year-round work.

8. Publish recent findings, and especially those from older work on biochemical characteristics of embryogenic wild carrot and incompetent loblolly pine cell suspensions.

RELATED STUDENT RESEARCH:

Completed Since Last Meeting.

Brent Earnshaw - Ph.D. Program, Biochemical Orientation, entitled "The Role of Cellular Anti-Oxidants (Glutathione and Ascorbic Acid) in the Growth and Development of Wild Carrot Suspension Cultures."

Rene Kapik - M.S., Independent Study, entitled "Phenolic Components of the Primary Cell Wall and Their Possible Role in the Regulation of Growth."

In Progress.

Russell Feirer - Ph.D. Program, Biochemical Orientation, investigating the role of polyamines and associated enzymes in plant development. In cooperation with the University of Wisconsin, Madison.

Luke Nealey - Ph.D. Program, Organic Chemistry Orientation, entitled "Isolation and Characterization of Xyloglucan from Suspension Cultured Loblolly Pine Cell Medium."

Note.

Efforts to attract IPC and other students are being intensified.

COOPERATIVE INVESTIGATIONS:

1. North Carolina State University - Cooperative evaluation with Dr. Ralph Mott and Dr. Henry Amerson of procedures for initiating embryogenic cultures of loblolly pine, Norway spruce, and white spruce.

2. St. Norbert College - A cooperative study with Dr. John Phythyon concerning techniques for isolating and characterizing DNA from conifer chloroplasts.

3. Yale University; Williams - A cooperative study with Dr. Robert Slocum who is assisting in efforts to characterize and localize ADC/ODS in cultured plant cells.

4. Ohio State University, Wooster - A joint effort with Dr. Howard B. Kriebel involving his constructing cloned cDNA sequences from embryogenic and nonembryogenic callus of Norway spruce. In addition, Dr. Kreibel has supplied immature white pine cones for our work on somatic embryogenesis.

ATTACHMENT NO. 1

CONCEPTUAL PLAN AND FLOW CHART